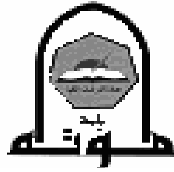


Mu´tah University
Deanship Of The Graduate Studies

Correlation Between Urinary Tract Infection In Diabetic and Non-Diabetic Patients In Ma'an Province, Jordan.

By
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A Thesis Submitted to the Deanship of the Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology, Department of Biology

Mu´tah University, 2013

Dedication

To my father ,who reap the thorns out of my way to pave me through science. To my mother, a symbol of love and healing balm. To my brothers and sisters, a thin pure hearts and souls of innocent.

To my wife, the pulse of heart, the artery of life and the rhythm of my time. To the joy of life, sun of days, moon of nights and all the rose in orchards, my children Kenan and Kenda.

Ali Mohammad Al-asoufi

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Ali Mohammad Al-asoufi

Table of Contents

List of Figures

List of Tables

Abstract

Correlation Between Urinary Tract Infection in Diabetic and Non Diabetic Patients in Ma'an Province, Jordan.

Ali Mohammad Al-Asoufi

Mu'tah University, 2013

Urinary tract infection (UTI) is the most infectious disease that affected both male and female. This study designed to detect the bacterial species that responsible for UTI in both diabetic patients and non-diabetic patients in Ma'an province, Jordan.

116 urine samples were investigated in order to determine UTI-causing bacteria. these samples distributed unequally between diabetic male(12) and diabetic female (25) and also non-diabetic male(13) and non-diabetic female (66). The results represent that *E.coli* is responsible for UTI in both diabetic and non-diabetic patients (15.5% and 29.3% respectively) with large proportion (44.8%).

This study showed that not all bacterial species that isolated from non-diabetic sample could be isolated from diabetic samples. *E. coli (15.5%), P. aeruginosa* (4.3%), *K. pneumonia* (1.7%), *P. mirabilis* (2.6%), *S. marcescens* (0.9%), *S. aureus* (1.7%), *S. pyogenes* (1.7%), *E. faecalis* (0.9%), *S. epidermidis* (1.7%) *and S. saprophyticus* (0.9%). But *E. aerogenes, E. cloacae, C. freundii, A. baumannii* and *B. subtilis* are five bacterial species that can't isolated from all diabetic samples

Our study show that for treatment of UTI in both diabetic and non-diabetic patients, Chloramphenicol (30 µg), Ciprofloxacin (5 µg) and Vancomycin (30 µg) are more favorable than other antibiotics. In the same time Cephalothin (30µg) is not recommended.

الملخص

العلاقة بين التهاب المسالك البولية للمرضى المصابين بالسكري وغير المصابين بالسكري ضمن محافظة معان، الأردن.

علي محمد العسوفي

جامعة مؤتة 2013

التهاب المسالك البولية من أكثر الأمراض المعدية والتي تصيب كلا الجنسين الذكور والإناث. هذه الدراسة صممت لتحديد أنواع البكتيريا المسببة لالتهاب المسالك البولية للمصابين بالسكري أو غير المصابين به.

تم تحصيل 116 عينة بول لتحديد أنواع البكتيريا علما بأن هذه العينات موزعة بشكل غير متساوي بين الذكور المصابين بالسكري (12) والإناث المصابات بالسكري (25) والذكور غير المصابين بالسكري (13) والإناث غير المصابات (66). هذه الدراسة بينت أن *E.coli* هي من مسببات التهاب المسالك البولية وبنسبة عالية عند المصابين بالسكري وغير مصابين به (15.5%، 29.3% بالترتيب) وبنسبة إجمالية (44.8%).

هذه الدراسة بينت أيضا أنه ليس كل أنواع البكتيريا التي عزلت من المرضى غير المصابين بالسكري ممكن عزلها من المصابين بالسكري. من الأنواع المشترك بينهما *E.coli*, ، *K.pneumonia* ، *P. mirabilis* ، *S. marcescene*، *S. aureus*، *S. pyogenes*، *E.faecalis*، *S. epidermidis* و *S. saprophyticus*. بينما لم يتم عزل كل من *E.aergenes*، *E. cloacae*، *C.freundii*، *A. baumannii* و *B.subtilis*.

تبين من الدراسة انه لعلاج التهاب المسالك البولية عند المصابين بالسكري وغير المصابين بالسكري يؤخذ كل من Chloramphenicol (30 µg)، Ciprofloxacin (5 µg) و Vancomycin (30µg). بينما Cephalothion (30µg) غير محبب وغير موصى به في جميع الحالات.

Chapter One
Introduction

1.1.Introduction

The urinary system or renal system is the system that produces, stores, and eliminates urine. In humans body it includes two bilateral kidneys, two ureters, single midline pelvic bladder and the urethra. The kidney function is to remove liquid waste from the blood in the form of urine, and also keeping a stable balance of salts and other substances in the blood. Ureters are small tubes which carry the urine from kidney to bladder. Urine is stored in the bladder and emptied out through the urethra. The female and male urinary system are similar, differing only in the length of the urethra (Ronald, 2003)

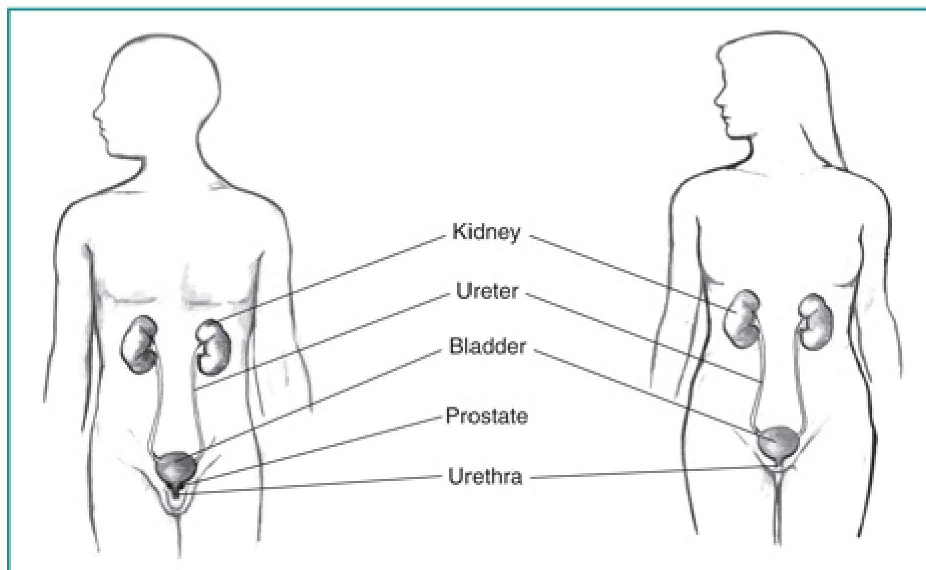


Figure 1
Male and female urinary system
(Kidney & Urology Foundation of America | 2 West 47th Street, Suite 401 | New York NY 10036 | 1.800.633.6628)

Urine is a liquid waste product of the body secreted by the kidneys by a process of filtration from blood called urination and excreted through the urethra. Urine contains a range of substances that vary with what is introduced into the body. Aside from water, urine contains an assortment of inorganic salts and organic compounds, including: urea $(NH_2)_2CO$, proteins, hormones, and a wide range of metabolites. Normally , the urine is sterile (free of microorganisms). So when the urine has microorganisms , at this case there is a urinary tract infection (Mansour et al., 2009).

Urinary tract infections (UTIs) are one of the most common infectious diseases which caused by different microorganisms. It's the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year (Daoud et al., 2011). The most common bacteria that cause UTI are gram negative bacteria as *Escherichia coli* and Gram positive bacteria as *Staphylococcus aureus*.

DM is a metabolic syndrome that characterized by increasing of blood glucose due to partially or completely lacking of insulin hormone . The patients with DM have dysfunctional bladder which lead to accumulation of urine in its pool which serves a good environment to the bacteria to be grow and cause UTI (Sibi et al., 2011).

Factors that have been proposed as constituting an enhanced risk for UTIs in diabetics include age, metabolic control, duration of DM, diabetic cystopathy, more frequent hospitalization and instrumentation of the urinary tract, recurrent vaginitis and vascular complications (Hoepelman et al., 2003).

In addition, a higher glucose concentration in the urine may create a culture medium for pathogenic microorganisms. Although the relation between diabetes and bacteriuria has been the subject of several controlled studies, the association between diabetes and UTI risk has not been examined until know (Boyko et al., 2005).

DM has long been considered to be a predisposing factor for UTI and the urinary tract is the principle site of the infection in diabetics with increased risk of complications of UTI. The most common cause of UTI in men and women with and without DM is *Escherichia coli*. In non-diabetic male and female, the frequency of organism causing UTI are: *Escherichia coli* 31.4% & 58.2%, *Enterococcus spp.* 9.4% & 6.5%, *Pseudomonas spp.* 17.2% & 4.7% respectively. The organisms causing UTI in diabetic female are *Escherichia coli* 54.1%, *Enterococcus spp.* 8.3%, *Pseudomonas spp.* 3.9%, while in diabetic male it is 32.5%, 9.4%, 8.5% respectively (Saber et al., 2010).

1.2.Aims of the study

This work was carried out in order to:

A. Determination the types of bacteria that cause urinary tract infection in both diabetic and non-diabetic patients in the population of ma'an province, Jordan.
B. Identification of bacterial species that isolated from diabetic samples and compared with the same isolated species from non-diabetic samples.
C. Detect the sensitivity of isolated bacteria to various antibiotics for both diabetic and non-diabetic patients.

Chapter Two
Review of Literature

2.2 Urinary Tract Infections

UTI are a common type of infection caused by bacteria (most often E. coli*)* that travel up the urethra to the bladder. A bladder infection is called cystitis. If bacterial infection spreads to the kidneys and ureters, the condition is called pyelonephritis (Ronald et al., 2001) Cystitis is considered a lower urinary tract infection. Pyelonephritis is an upper urinary tract infection and is much more serious.

2.2.1 Symptoms

Strong urge to urinate frequently, even immediately after the bladder is emptied, painful burning sensation when urinating, discomfort, pressure, or bloating in the lower abdomen, pain in the pelvic area or back, cloudy or bloody urine, which may have a strong smell

2.2.2 Types of UTIs.

UTIs are generally classified as (Mansour et al., 2009):

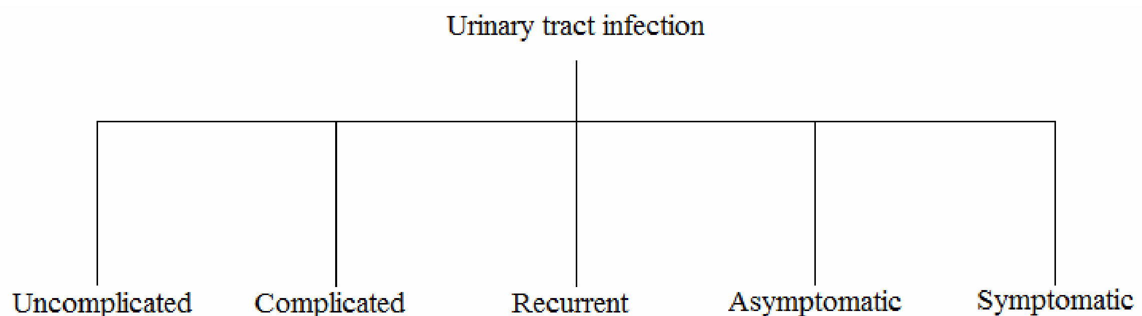


Figure 2
Types of urinary tract infection

2.1.2.1 Uncomplicated Urinary Tract Infections

Uncomplicated UTIs are due to a bacterial infection, most often *E. coli*. They affect women much more often than men (Mansour et al., 2009). Examples:- *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella spp.* and *Enterococcus faecalis.*

2.1.2.2 Complicated Urinary Tract Infections

Complicated infections, which occur in men and women of any age, are also caused by bacteria but they tend to be more sever, more difficult to treat, and recurrent (Mansour et al., 2009) .Examples:-

Escherichia coli, *Klebsiella spp.*, *Enterobacter cloacae*, *Serratia marcescens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and Group B *streptococci.*

2.1.2.3 Recurrent Urinary Tract Infections

Most women who have had an uncomplicated UTI have occasional recurrences. About 25 - 50% of these women can expect another infection within a year of the previous one. Between 3 - 5% of women have ongoing, recurrent urinary tract infections, which follow the resolution of a previous treated or untreated episode (Gopal et al., 2007).

2.1.2.4 Asymptomatic Urinary Tract Infection

When a person has no symptoms of infection but significant numbers of bacteria have colonized the urinary tract, the condition is called asymptomatic UTI (also called asymptomatic bacteriuria). The condition is harmless in most people and rarely persists, although it does increase the risk for developing symptomatic UTIs (Aguirre-Avalos et al., 1999).

2.2.2.5 Symptomatic Urinary Tract Infection

When a patients with a positive urine culture and experiencing the symptoms of UTI according to the presence of bacteria, the condition is called symptomatic UTI and also called symptomatic bacteria (Takahashi et al., 2004).

2.1.3 Epidemiology

Incidence, females: 1,200 cases per 100,000 persons annually. males: 30 cases per 100,000 persons annually. Prevalence: Females: 1,000 to 4,000 cases per 100,000 persons, males: <100 cases per 100,000 persons, asymptomatic bacteriuria occurs in up to 40% of elderly men and women (Takahashi et al., 2004).

Age, among infants up to 6 months of age, UTI is more common in boys, among persons between 1 and 65 years of age, predominantly occurs in female patients, among persons over age 65, affects men and women roughly equally (approximately 40%) (Takahashi et al., 2004).

Gender, most prevalent in sexually active women, women over age 65, or without estrogen, also have more frequent UTIs than men. Genetics: Patients who do not secrete ABO antigens are three to four times more likely to have UTIs (Takahashi et al., 2004).

2.3 Diabetes Mellitus

DM describes a metabolic disorder of multiple a etiology characterized by chronic hyperglycemia with disturbances of

carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss (Boyko et al., 2005).

2.3.1 Diabetes Mellitus Type 1

Also called insulin dependent DM , indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death. It happens most often in children and young adults but can appear at any age (Ramirez et al., 1989)

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2.3.2 Diabetes Mellitus Type 2

Also called non-insulin dependent DM, is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature (Jimenez et al., 2012).

2.3.3 DM and UTI

UTI is a significant problem in patients with DM because of the multiple effects of this disease on the urinary tract and host immune system. Complicated UTIs associated with diabetes include renal and peri-renal abscess, gas forming infectious, such as emphysematous pyelonephritis and emphysematous cystitis, fungal infections and renal papillary necrosis (Patterson et al., 1997).

Complicated UTIs occur most commonly in patients with abnormalities of the genitourinary tract. However, other conditions such as age over 65 years, treatment with immunosuppressive drugs, the presence of human immunodeficiency virus-infection and diabetes mellitus (DM) also predispose to an enhanced susceptibility for the development of a UTI with a complicated course.

Diabetic patients also suffer more frequently from complicated infections compared with non-diabetic patients. In a large study of bacteraemic patients, it was demonstrated that two thirds of the patients had DM; the urinary tract was the most prevalent infection site (Carton et al., 1992).

Diabetic patients are at a higher risk developing acute pyelonephritis, renal abscess, abnormalities of bladder scarring and pyelitis (Boyko et al., 2005). People with diabetes have dysfunctional bladders which contract poorly. Women are prone to UTIs for reasons which are not well understood. Every one woman develops UTI among

five women. UTI is uncommon in men and contributes to have larger complications after initial infection (Carton et al., 1992).

2.4 Expected isolated bacteria from urinary tract infection

2.3.1 *Escherichia*

Escherichia is a genus of gram-negative, facultative anaerobic, non-spore forming, that belong to *Enterobacteriaceae* family rod-shaped bacteria .While many *Escherichia* are harmless commensals, particular strains of some species are human pathogens, and are known as the most common cause of urinary tract infections, significant sources of gastrointestinal disease (Ronald, 2003).

Escherichia coli is a common bacterium characteristically found in the human and animal intestine. The bacteria form part of the normal gut flora present in the bowel. There are a number of different types of *E. coli*, the majority of which are harmless although a few strains of the bacteria can cause serious food poisoning and infections. A common infection caused by the *E. coli* bacteria is cystitis which is an infection of the bladder caused when the *E. coli* bacteria spreads from the gut to the urinary tract. In general, women are more susceptible to this *E. coli* infection explained by the biological fact that the urethra and anus lie in close proximity in women (Daoud & Afif, 2011).

E.coli which associated with both Urinary tract infection and gastrointestinal illness have two main important serotypes O157 and H7 (Khleifat et al., 2006). The "O" in the name refers to the cell wall (somatic) antigen number, whereas the "H" refers to the flagella antigen.

Generally wild type *E.coli* and *E.coli* O175:H7 are pathogenic and cause UTI, but both of them are different in surface charge , so the mode of action between these bacteria and the environment will be differ (Khleifat et al., 2006).

2.3.2 *Klebsiella*

Klebsiella is a gram negative bacteria, encapsulated, lactose fermenting bacteria, facultative anaerobic, rod shaped, non-motile bacteria, the most important species is *K. pneumonia*e.

K. pneumoniae is a bacterial organism that is responsible for causing pneumonia, sepsis, and UTI. The organism resides in the upper respiratory tract and gastrointestinal tract of healthy individuals. It causes infection of the urinary tract when it is introduced to the region by spread of fecal matter containing the organism(Lauet et al., 2007). UTI from *K. pneumoniae* can be prevented by removing indwelling catheters as soon as they are no longer needed and reducing exposure to infectious organisms in those who are predisposed to infection.

2.3.3 *Enterobacter*

Enterobacter is a genus of common Gram-negative, rod-shaped, facultative anaerobic bacteria, of the family *Enterobacteriaceae*. The urinary and respiratory tracts are the most common sites of infection. They are the most important nosocomial pathogen (Al Ansari et al., 1994) . These bacteria contain β-lactamases, which are very difficult to be detected in lab, and its presence is responsible for resistance during treatment (Khleifat et al., 2006).

2.3.3.1 *Enterobacter cloacae*

E. cloacae are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infection, skin and soft tissue infections, urinary tract infections, endocarditic. mainly isolated as nosocomial infections in the ICU (Intensive-care unit) for those who stay in the hospital for prolonged periods (Juanjuan et al., 2007).

2.3.3.2 *Enterobacter aerogenes*

Infections commonly attributed to *E. aerogenes* are respiratory, gastrointestinal, and urinary tract infections, specifically cystitis, in addition to wound, bloodstream, and central nervous system infections. E. *aerogenes* isolated as nosocomial infection as *E. cloacae* (Chevalier et al., 2004).

2.3.4 *Proteus*

Proteus is gram-negative, rod-shaped bacterium within the *enterobacteriaceae* that can be found as part of the micro flora in the human intestine. This organism is not usually a pathogen, but does become a problem when it comes into contact with urea in the urinary tract (Chen et al., 2012). The most common species of this genus that cause UTI is *P. mirabilis*

2.3.4.1 *Proteus mirabilis*

Proteus mirabilis is not a common cause of UTI in the normal host, but its most often associated with urinary stones (due to presence of urease enzyme) and catheter encrustation and is rarely a cause of acute cystitis, recurrent UTI, but is more often associated with complicated UTI (Li & Mobley, 2002).

P. mirabilis has a peritrichous flagella that facillate them to spread through urinary system and elongated from single cells to multi-elongated cells and caused UTI.

P. mirabilis strains are resistant to amoxicillin, penicillin, fluoroquinolones and other broad-range activity antibiotics, so UTI that

caused by this bacteria may be became lethal in long-term hospital patients (Cokeret al., 2000).

2.3.5 *Serratia*

Serratia is a genus of gram negative , rod shaped and facultative anaerobic bacteria , belong to *enterobacteriaceae*. There are multi-species of this genus , but *S. marcescens* is normally the only pathogen and usually causes nosocomial infections (Maki et al., 1973).

2.3.5.1 *S. marcescens*

S. marcescens is commonly found growing in bathrooms due to its ubiquitous presence in the environment, and its preference for damp conditions. It is produces a reddish-orange pigment called prodigiosin, so it cause extrinsic staining of the teeth (Su et al., 2003).

S. marcescens is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections (Su et al., 2003).

2.3.6 *Pseudomonas*

Pseudomonas is a gram negative bacteria, coccobacillus bacterium of relatively low virulence. *Pseudomonas* is strictly aerobic bacteria. It may be found in soil, water, plants, and animals.(Liang et al., 2001).

The current classification of the genus *Pseudomonas* is divided into five groups based on ribosomal RNA (rRNA)/DNA homology. More than 20 pseudomonal species that have been found from human clinical specimens, the following four representative species are the most common: *Pseudomonas aeruginosa, Pseudomonas cepacia, Pseudomonas pseudomallei, and Pseudomonas mallei.*

Pseudomonas aeruginosa colonization reportedly occurs in more than 50% of humans, and *P aeruginosa* is the most common pseudomonal species (Bitsori et al., 2012).

2.3.6.1 *P. aeruginosa*

More than half of all clinical isolates produce the blue-green pigment phyocyanin. *Pseudomonas* often has a characteristic sweet odor (Mittal et al., 2009).

P. aeruginosa has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs). Pseudomonal infections are complicated and can be life threatening (Bitsori et al., 2012).

P. aeruginosa is the third most common (after *E.coli , p. mirabilis*) pathogen associated with hospital-acquired catheter associated UTIs, and also may cause meningitis and respiratory tract infection in patients with cystic fibrosis (Hoepelman et al., 2003).

2.3.7 *Enterococcus*

Enterococcus are Gram-positive cocci that often occur in pairs (diplococci) or short chains, facultative anaerobic organisms. *Enterococcus* are part of the normal intestinal flora of humans and animals but are also important pathogens responsible for serious infections. They have a fermentative metabolism in which they convert carbohydrates to lactic acid, so that *Enterococcus* is a genus of lactic acid bacteria (Murray, 1990).

2.3.7.1 *E. faecalis*

E. faecalis is a non-motile, facultative anaerobic microbe, it ferment glucose without gas production, catalase negative. It can cause cause endocarditis and bacteremia, urinary tract infections, meningitis.

E. faecalis is a bacteria naturally found in soil, water and plants. It is also produced in the bodies of humans and some animals. Some forms of this bacteria, like the type found in fecal matter, can be extremely harmful to humans (Guzman et al., 1989). *Enterococcus faecalis* is an infection that affect the gastrointestinal tract and is mostly found in hospitals and other health-care institution.

Enterococcus faecalis can cause pain or pressure during urination, usually categorized as a urinary tract infection (UTI). It can also cause conditions such as endocarditic. Other side effects of this disease are wound infections, blood infections and pelvic infections (Domig et al., 2003).

2.3.8 *Staphylococcus*

Staphylococcus is gram positive bacteria, coccal in shaped, catalase positive facultative anaerobic,. It may be coagulase positive as *S. aureus* or coagulase negative as *S. epidermidis* (Ronald, 2003).

2.3.8.1 *S. aureus*

S. aureus is a type of bacteria. It stains gram positive and is non-moving small round shaped or non-motile cocci. It is found in grape-like (staphylo-) clusters. This is why it is called *Staphylococcus*.

S. aureus belongs to the family *Staphylococcaceae*. It affects all known mammalian species, including humans (Baba-Moussa et al., 2008). *S. aureus* is transmitted through air droplets or aerosol and also

through direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals.

S. aureus causes superficial skin lesions such as boils, styes and furuncles; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections

S. aureus is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices (Baba-Moussa et al., 2008).

2.3.8.2 *S. saprophyticus*

S. saprophyticus is a gram positive and coagulase-negative species of *Staphylococcus* bacteria and often implicated in urinary tract infections. *S. saprophyticus* colonizes in the urinary tract of young women and men of all ages. In females between the ages of about 17-27. *S. saprophyticus* is the second most common causative agent of acute UTIs, after *Escherichia coli* (Goldenring, 1986). The bacteria may also reside in the urinary tract and bladder of sexually active females.

S. saprophyticus contains urease, which hydrolyzes urea and produces a derivative of ammonia. This is how the cell metabolizes nitrogen. Urease activity is known to be an infection causing factor in UTIs (Aguirre et al., 1999).

Patients with UTIs caused by *S. saprophyticus* usually present with symptomatic cystitis (Hedman et al., 1991). The urine sediment of a patient with UTI caused by *S. saprophyticus* has a characteristic appearance under the microscope.

2.3.8.3 *S. epidermidis*

Catalase-positive, coagulase-negative, facultative anaerobe, gram positive bacterium . consisting of non-motile, cocci, arranged in grape-like clusters. It is part of human skin flora, and also be found in the mucous membranes and in animals.

These bacteria are responsible for a growing number of infections among hospital patients whose immune systems are weakened (Hedman et al., 1991), so it will cause urinary tract infection of indwelling urinary catheters or urinary tract complications (Hall et al., 1994).

A characteristic of many strains of this microbe is the production of a capsule or slime resulting in the formation of a biofilm. In a biofilm, *S. epidermidis* is protected against attacks from the immune system and against antibiotic treatment, making *S. epidermidis* infections difficult to stop (Hall et al., 1994).

2.3.9 *Streptococcus*

Streptococci is a Gram-positive, non-motile, non-sporeforming coccus that occurs in chains or in pairs of cells. *Streptococci* are oxidase- and catalase-negative (Khleifat et al., 2006).

Species of *Streptococcus* are classified based on their hemolytic properties, Alpha hemolytic species cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar. Beta hemolytic species cause complete rupture of red blood cells. On blood agar, this appears as wide areas clear of blood cells surrounding bacterial colonies. Gamma-hemolytic species cause no hemolysis (Terao, 2012).

2.3.9.1 *S. pyogenes*

S. pyogenes, also known as Group A *Streptococcus* (GAS), is the causative agent in a wide range of Group A streptococcal infections, that belong to Beta-hemolytic *Streptococcus*. These infections may be non-invasive or invasive (Terao, 2008).

S. pyogenes is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Urinary tract infection and pharyngitis ("strep throat") are an examples of *S. pyogenes* infections, and localized skin infection ("impetigo").

2.3.10 *Citrobacter*

Gram-negative bacteria that belong to *Enterobacteriaceae* family which are found in water, soil, and as commensals within the human gastrointestinal tract. *Citrobacter* species are differentiated by their ability to convert tryptophan to indole, ferment lactose, and use malonate. They are rarely the source of illnesses, except for infections of the urinary tract and infant meningitis and sepsis (Kus et al., 2007).

The role of *Citrobacter* species in human disease is not as great as that of the other coliforms and *Proteus*. *C. freundii* and *C. diversus* have been isolated predominantly as super-infecting agents from urinary and respiratory tract infections (Kus et al., 2007).

2.3.11 *Acinetobacter*

Acinetobacter is a genus of Gram-negative bacteria. This bacteria are non-motile and oxidase-negative, coccobacillus, strictly aerobic, encapsulated, and non-fermentative. (Braun et al., 2004). *Acinetobacter* are a key source of infection in debilitated patients in the hospital, in particular the species *A. baumannii*.

Acinetobacter are widely distributed in nature, and commonly occur in soil. They can survive on moist and dry surfaces, including in a

hospital environment. So *Acinetobacter* is frequently isolated in nosocomial infections, and is especially prevalent in intensive care units (Braun et al., 2004).

One of the most striking features of *Acinetobacter spp*. is their extraordinary ability to develop multiple resistance mechanisms against several major antibiotic classes due to presence of resistance marker in its plasmid (Levin et al., 1999).

2.3.12 *Bacillus*

Bacillus is Gram-positive bacteria that has rod-shaped ,facultative anaerobes, catalase positive, endospores forming bacteria.

Bacillus subtilis is one of the strains that cause urinary tract infection, but its usually prevalent and resistance to many types of antibiotics. Although *B. subtilis* used as probiotic in healthy individuals but can be cause disease in immunocompromised patients (Gul et al., 2004).

Chapter Three
Material and Methods

3.1 Collection of Samples

In this study , one hundred sixteen patients were subjected from different medical center from ma'an province, Jordan. These patients have symptoms of urinary tract infection.

All the samples were collected within three months from 5 to 51 years old patients of both male and female and diabetic or non-diabetic . All these patients did not receive any antimicrobial therapy for several weeks before sampling.

The samples were collected at morning using a sterile urine container which open just in the sampling process to prevent any contaminations. The samples transported to the laboratory to culture them on a suitable media for 24 hr's under aseptic techniques, and storage the samples at 4^{o}C for further study.

3.2 Isolation of Bacteria

3.2.1 Culture Media

1. Nutrient Broth: This medium consists of: (5g) Peptic digest of animal tissue, (5g) sodium chloride, (1.5g) beef extract and (1.5g) yeast extract dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min. For Nutrient agar (15g) of agar was added with Nutrient broth
2. Nutrient Agar: This medium consists of: (5g) Peptic digest of animal tissue, (5g) sodium chloride, (1.5g) beef extract , (1.5g) yeast extract and 15g of agar dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min.
3. MacConkey agar: (20g) Peptic digest of animal tissue, (10g) lactose, (5g)bile salts, (5g)sodium chloride, (0.075g)neutral red and (15g) agar dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min.
4. Mannitol Salt Agar: (10g) Peptic digest of animal tissue, (1g) beef extract , (10g) D-mannitol, (75g) sodium chloride, (0.025g) phenol red and (15g) agar dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min.
5. Eosin Methylene Blue Agar: (10g) Peptic digest of animal tissue, (5g) lactose, (5g) sucrose, (2g) dipotassium phosphate, (0.065g) methylene blue, (0.4g) eosin Y and (15g) agar dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min.

6. Blood Agar (Azide): (10g) tryptose, (3g) meat extract, (5g) sodium chloride, (0.2g) sodium azide and (15g) agar dissolved in /1 liter of distilled water. pH was adjusted to 7.4 autoclaving at 121°C for 15 min. After autoclaving and cooling to 50^oC , human blood (7%) was added to the media under aseptic condition.

3.2.3 Inoculation of Media

Upon arrival the samples to the laboratory, they were cultured on the nutrient agar to be sure that these samples were infected with UTI. The bacteriological loop firstly flamed then cooled and dipped into the urine and streak numerous times along the enteric plate.

The pure colony that resulted from the first inoculation was cultured into four plates of macConkey agar, Mannitol salt agar, Eosin Methylene blue and Blood agar to selective and differentiate the resulted colony.

All these five agar were incubated aerobically at 37^oC and checked after 24 hr's and 48 hr's.

3.3 Morphological Identification

The isolated pure colony from nutrient agar were examined under dissecting microscope to detect many morphological categories which include:

Shape of colony (form), edge of colony (margin), color of colony, elevation of colony, odor of colony and viscosity of colony, are shown in figure 3

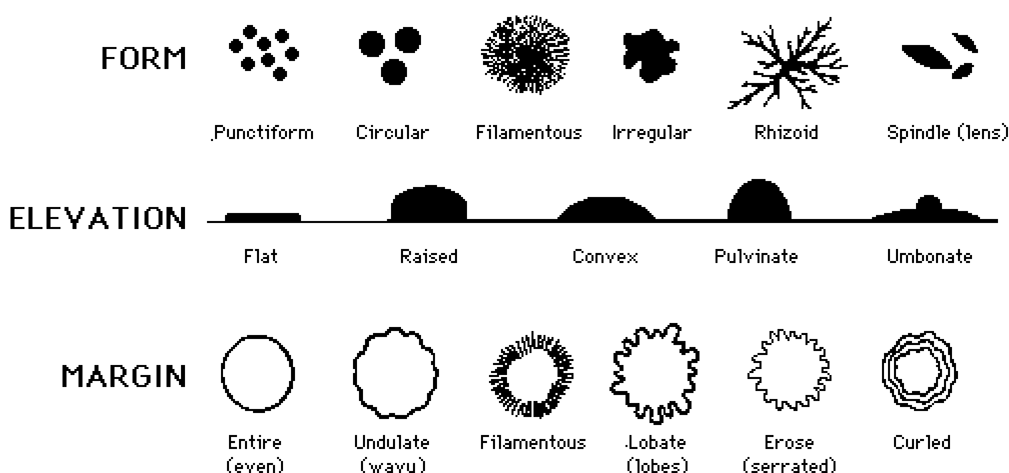


Figure 3
Bacterial colonies morphology
(Michael T. and John M. (2006))

3.4 Identification of Isolated Bacteria

3.4.1 Staining Procedure

3.4.1.1 Gram Stain

In the Gram stain, the cells were first heat fixed and then stained with a basic dye crystal violet, and left to react to 1 minute, which is taken up in similar amounts by all bacteria. The slides were then treated with an iodine solution (mordant) for also 1 minute to fix the stain , washed briefly with 95% alcohol (destained) by dipping the smear few seconds, after that the slide washed with tap water. Finally counterstained with a counter satin safranin for 1 minute and then washed with tap water, dried and examined under oil immersion lens of compound microscope.(Wiwanitkit et al., 2005)

All stains were filtered before use. Crystal violet consists of two solutions: Solution A:2g of crystal violet dissolved in 20 ml of 95% ethanol. Solution B: 0.8 g of ammonium oxalate dissolved in 80 ml of distilled water. Solution A and B mixed together to be used as crystal violet solution.

Iodine was prepared by dissolving 2 g of potassium iodide (KI) in 300 ml of distilled water, then 1 g of finely grounded iodine crystals added and stirred at room temperature until completely dissolved.

Safranin was also prepared by dissolving 1 g of safranin in 100 ml of distilled water.

3.4.1.2 Capsule Stain

Capsule stains are not heat-fixed, and water is never used to rinse, because capsules are highly ordered polymers of sugars and proteins. The primary stain applied is crystal violet, which stains both the bacterial cell and the surrounding capsule. A copper sulfate solution is then applied, which serves a dual function as both decolorizer and counter stain. It removes and replaces the crystal violet in the capsule only. Finally the slide was focused using oil immersion lens of compound microscope (Petras et al., 1985).

Crystal violet as primary stain was prepared as in Gram stain section. The copper sulfate solution was prepared by dissolving 20g of copper sulfate crystals in 100ml d.H_2O.

3.4.1.3 Endospore Stain

Bacterial smear were heat fixed and placed over a steam bath and covered with Malachite Green and incubated over the bath for 3 - 5 minutes. After that the stain was removed and allow the slide to dry and rinsed with tap water. The smear was covered with safranin for 2 minutes and then washed with tap water. Finally the slide was examined under oil immersion lens of compound microscope (Petras et al., 1985).

Primary stain was prepared by dissolving 0.5 g of malachite green powder in 100 ml of distilled water. While the decolorizing agent is tap water.

Safranin as counter stain was prepared by dissolving 2.5 g of safranin O in 100 ml of 95% ethanol, after that this solution was diluted by dissolved 10 ml of stock solution with 90 ml of distilled water.

3.4.1.4 Flagella Stain

Bacterial smear for flagella stain should be very young culture (about 6 to 12 hours) to avoid loss of flagella. Firstly the slide cleaned with 95% ethanol and wait for dry to removed any contaminants. About 5ml to 10ml of culture putted at one end of slide, the using another clean spreader the culture was spreaded to other end of slide, and allowed to air-dry without heat fixation. The slide then was flooded completely with Leifson stain for 10 minutes and washed with tap water. Finally the slide was air-dried and examined using oil immersion lens of compound microscope (Wiwanitkit et al., 2005).

Leifson stain consist of three solutions, solution A prepared by dissolving 1.5g of sodium chloride in 100 ml distilled water . solution B prepared by dissolving 3g of tannic acid in 100 ml distilled water , solution C prepared by dissolving 0.9g of pararosaniline acetate and 0.3g of pararosaniline hydrochloride in 100ml Ethanol, 95% . after that equal volumes of solutions A and B was mixed then added 2 volumes of the mixture to 1 volume of solution C.

3.4.2 Oxidase Test

Oxidase test was used to determine if a bacterium produces certain cytochrome c and the enzyme cytochrome oxidases as a part of their respiratory chain. These bacteria can therefore utilize oxygen for energy production with an electron transfer chain.

This test can be performed within second using a specific strips that impregnated with N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochlorid, cytochrome oxidase oxidizes cytochrome c which in turn oxidizes the N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochlorid producing a dark blue/violet colored product.

Gently touch the colonies that will be tested with oxidase detection strip or remove a colonies to clean slide by a sterile loop and touch the strip to them. After that the result shown within 30 second, the development of dark blue/violet color indicates an oxidase positive otherwise oxidase negative (Tarrand et al., 1982).

3.4.3 Catalase Test

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. It is easy to test for this enzyme in bacteria. A test culture is exposed to 3% H_2O_2. If catalase is present, H_2O_2 is broken down to H_2O and O_2 .The oxygen is detected as a steady evolution of gas bubbles from the culture.

Firstly transfer a large isolated colony from the culture dish to a microscope slide. After that two drops of (3%) hydrogen peroxide reagent (H_2O_2) was added to the colony. If the air bubbling (gas) was produced, the bacteria is catalase positive, if there was no bubbles, the bacteria is catalase negative (Taylor et al., 1972).

3.4.4 Coagulase Test

This test used to differentiate *Staphylococci aureus* from other genus of coagulase negative S*taphylococci*.

The purpose of the coagulase test is to determine whether a bacterium produces coagulase, an enzyme capable of coagulating liquid plasma into a solid clot. This test can be performed by two methods, slide method (for bounded coagulase enzyme) and tube method (for free coagulase enzyme)

In the tube method, loopful of tested organism was taken from broth culture and added to the tube which contain a plasma, here should be have a negative control tube by added a culture to the empty tube or normal saline to plasma tube. The negative control very important to compare the result.

So if the coagulase enzyme is found the clot appears within half hour and the test is positive , but if there are no clot that is mean no coagulase enzyme so the test is negative.

In slide method, also should be have two circles on the slide, one for the tested culture and another for negative control. The test can be summarized by taken one drop of sterile saline into each circle, after that emulsifying the tested colonies in saline in the first circle while another circle used as free of culture. Few drops of plasma (undiluted) was added to each circle and the results will seen within few minutes.

If a clotting seen in the form of plasma clumping so coagulase test is positive otherwise coagulase test is negative (Berke et al., 1986).

3.4.5 Microgen GN -ID System

The Microgen GN A-ID comprises of only 12 substrates which are specifically selected to optimize the identification of the most commonly encountered oxidase negative Bacilli including the family *Enterobacteriaceae* and *Acinetobacter spp.*

Combination of Microgen GN A + GN B identification systems used for the identification of the commonly encountered *Enterobacteriaceae* from urinary samples that oxidase-positive gram negative Bacilli.

The bacterial culture were examined by gram stain and oxidase test prior to use of the Microgen GN -ID System.

3.4.6 Microgen Bacillus-ID System

Microgen Bacillus-ID has been developed for the identification of *Bacillus spp* and related genera. Simple and easy-to-use 24 reaction system and the results was examined in 48 hours.

The bacterial culture were examined by oxidase test prior to use of the Microgen Bacillus-ID System.

3.4.7 Microgen Strep-ID

Microgen Strep-ID is a biochemical test system which utilizes a 12 well (12 test) microwell test strip and 3 off-strip tests; hippurate hydrolysis (provided), alpha-hemolysis and beta-hemolysis for the identification of Streptococcal and Enterococcal species.

Substrates that used were selected specifically for differentiate between *Streptococcus*, *Enterococcus* and related species by simple and easy method, and the results in 24 hours.

3.4.8 Microgen Staph-ID

Microgen Staph-ID has been developed for the identification of commonly encountered *Staphylococcus spp*. Gram stain (positive), catalase (positive) and latex agglutination / coagulase tests are performed as pre-tests on the isolate.

Substrates that used were selected specifically for *Staphylococcus* and related species by simple and easy method and the results in 24 hours.

3.5 Sensitivity Test

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection.

This test was performed according to disc diffusion method. By using series of antibiotic-disk that placed on the muller-hinton agar media which inoculated to form a bacteria lawn. The plate was incubated with bacteria at 37^{o}C for 24 hr's. If the organism is susceptible to antibiotic, a clear zone appears around the disk where growth has been inhibited.

The inhibition zone depends on the sensitivity of the bacteria to the specific antibiotics and also the antibiotic diffusion through the agar (Gul et al., 2004).

The antibiotic that used is the following : Ciprofloxacin, Gentamicin, Nalidixic , Ampicillin, Amoxicillin, Chloramphenicol, Tetracycline, Vancomycin, Cefuroxime and Cephalothin.

Chapter Four
Results, Discussions and Recommendations

4.1 Results

One hundred sixteen patients were of both diabetic or not-diabetic was summarized as in table 1. And these patients also differ in age from 5 years to 51 years. (Table 2)

Table 1
Sex and number of diabetic and non-diabetic patients with UTI

Gender	Male %	Female %
	25 (21.5)	91 (78.5)
Not-diabetic	13 (16.5)	66 (83.5)
Diabetic	12 (32.4)	25 (67.6)

Table 2
The age and sex distribution of diabetics and non-diabetic patients with UTI.

Age (year)	Female Non-Diabetic	Male Non-Diabetic	Female Diabetic	Male Diabetic
5 to 10	6 (9.09%)	0 (0.0%)	0 (0.0%)	3 (25.0%)
11 to 20	10 (15.15%)	5 (38.46%)	0 (0.0%)	0 (0.0%)
21 to 30	19 (28.79%)	4 (30.77%)	6 (24.0%)	0 (0.0%)
31 to 40	24 (36.36%)	3 (23.08%)	2 (8.0%)	2 (16.67%)
41 to 50	7 (10.61%)	1 (7.69%)	11 (44.0%)	7 (58.33%)
51 to 60	0 (0.0%)	0 (0.0%)	6 (24.0%)	0 (0.0%)
Total	66 (56.9%)	13 (11.21%)	25 (21.55%)	12 (10.34%)

4.1.1 Morphological Identification

Microscopical examination and many stain procedures including gram stain , capsule stain, endospore stain and flagella stain of all 116 samples was revealed that there are both gram positive and gram negative bacteria , and also there are a lot of types of bacteria due to different morphological studies. These UTI-causing bacteria shown in Table 3.

Table 3
Bacterial species that were isolated from patients affected with UTI.

Bacterial isolated	Percentage
E. coli	44.82%
P. aeruginosa	7.76%
K. pneumonia	6.90%
E. aerogenes	3.45%
E. cloacae	1.72%
P. mirabilis	10.34%
S. marcescens	1.72%
C. freundii	0.86%
A. baumannii	0.86%
S. aureus	5.17%
S. pyogenes	2.59%
E. faecalis	6.03%
S. epidermidis	3.45%
B. subtilis	1.72%
S. saprophyticus	2.59%

Table 4
Sex, cell shape, colony appearance and colony color for bacterial cells that isolated from diabetic and non-diabetic UTI samples

Isolate #	Bacterial Isolated	Morphology	Colony Appearance				Colony color
			Form	Elevation	Margin	Appearance	
1	*A. baumannii*	Rods	Circular	Mucoid	Entire	Smooth	Slightly Pink
2	*B. subtilis*	Rods	Circular	Flat	Undulate	Rough	Milky
3	*C. freundii*	Rods	Circular	Convex	Entire	Rough	Slightly Pink
4	*E. aerogenes*	Rods	Circular	Convex	Entire	Rough	Pinkish
5	*E. cloacae*	Rods	Circular	Convex	Entire	Rough	Slightly Pink
6	*E. coli*	Rods	Irregular	Convex	Circular	Rough	Pinkish
7	*E. faecalis*	Cocci in clusters	Circular	Convex	Entire	Smooth	Milky
8	*K. pneumonia*	Rods	Circular	Mucoid	Entire	Rough	Slightly Pink
9	*P. aeruginosa*	Rods	Circular	Convex	Entire	Rough	Green
10	*P. mirabilis*	Rods	Circular	Convex	Entire	Rough	Slightly Pink
11	*S. aureus*	Cocci in clusters	Circular	Convex	Entire	Smooth	Yellow
12	*S. epidermidis*	Cocci in clusters	Circular	Convex	Entire	Smooth	White
13	*S. marcescens*	Rods	Circular	Convex	Entire	Rough	Red
14	*S. pyogenes*	Cocci in chain	Circular	Convex	Entire	Rough	Grayish-white
15	*S. saprophyticus*	Cocci in clusters	Circular	Convex	Entire	Smooth	White

Table 5
Gram stain, presence of capsule and spore and flagella of isolated bacteria from diabetic and non-diabetic UTI samples.

Isolate #	Bacterial Isolate	Gram Stain	Capsule Stain	Endospore Stain	Flagella Stain
1	*A. baumannii*	Gram Negative	Capsulated	Non-spore former	Un-flagellated
2	*B. subtilis*	Gram Positive	Capsulated	Spore former	Monotrichous
3	*C. freundii*	Gram Negative	Non-capsulated	Non -spore former	Peritrichous
4	*E. aerogenes*	Gram Negative	Capsulated	Non -spore former	Monotrichous
5	*E. cloacae*	Gram Negative	Capsulated	Non -spore former	Peritrichous
6	*E. coli*	Gram Negative	Capsulated	Non -spore former	Peritrichous
7	*E. faecalis*	Gram Positive	Capsulated	Non -spore former	Peritrichous
8	*K. pneumonia*	Gram Negative	Capsulated	Non -spore former	Peritrichous
9	*P. aeruginosa*	Gram Negative	Capsulated	Non -spore former	Peritrichous
10	*P. mirabilis*	Gram Negative	Capsulated	Non -spore former	Peritrichous
11	*S. aureus*	Gram Positive	Capsulated	Non -spore former	Un-flagellated
12	*S. epidermidis*	Gram Positive	Capsulated	Non -spore former	Un-flagellated
13	*S. marcescens*	Gram Negative	Capsulated	Non -spore former	Peritrichous
14	*S. pyogenes*	Gram Positive	Capsulated	Non -spore former	Un-flagellated
15	*S. saprophyticus*	Gram Positive	Capsulated	Non -spore former	Un-flagellated

4.1.2 Biochemical Characteristic

Based on the above morphological and stained studies and biochemical characteristic such as catalase test, oxidase test, urease test, coagulase test and another test that included in Microgen kit, all the samples was identified on both diabetic and non-diabetic samples of both male and female patients (Table 6).

All the samples were divided into five groups depending on the results that illustrate in table 6. First group include gram positive bacilli bacteria that treated with Microgen Bacillus-ID Kit . Second group is gram negative oxidase negative bacteria that treated with Microgen GN A -ID Kit. Third group is gram negative oxidase positive bacteria that treated with Microgen GN A+B-ID Kit. Fourth group is gram positive cocci in chain that will be treated with Microgen Strep-ID Kit. The last group is gram positive cocci in cluster that will be treated with Microgen Staph-ID Kit (Table 7).

4.1.3 Sensitivity Test

All isolated bacteria were tested using different discs of antibiotic using a procedure which previously discussed to checked up of antibiotic resistance profile for diabetic-UTI and non-diabetic-UTI patients (Table 8 and Table 9).

Table 6
Oxidase test, catalase test, coagulase test, urease test and hemolysis pattern of bacterial isolated from diabetic and non-diabetic UTI samples.

Isolate #	Bacterial Isolate	Oxidase Test	Catalase Test	Coagulase Test	Hemolysis Pattern	Urease test
1	*A. baumannii*	Negative	Positive	Negative	γ	Negative
2	*B. subtilis*	Positive	Positive	Negative	β	Negative
3	*C. freundii*	Negative	Positive	Negative	γ	Positive
4	*E. aerogenes*	Positive	Positive	Negative	γ	Positive
5	*E. cloacae*	Negative	Positive	Negative	γ	Negative
6	*E. coli*	Negative	Positive	Negative	γ	Negative
7	*E. faecalis*	Negative	Negative	Negative	γ	Negative
8	*K. pneumonia*	Negative	Positive	Negative	γ	Positive
9	*P. aeruginosa*	Positive	Positive	Negative	β	Negative
10	*P. mirabilis*	Negative	Positive	Negative	γ	Positive
11	*S. aureus*	Negative	Positive	Positive	β	Positive
12	*S. epidermidis*	Negative	Positive	Negative	γ	Positive
13	*S. marcescens*	Negative	Positive	Negative	γ	Positive
14	*S. pyogenes*	Negative	Negative	Negative	β	Negative
15	*S. saprophyticus*	Negative	Positive	Negative	γ	Positive

Table 7
Distribution of isolated bacteria in the diabetic and non-diabetic UTI samples for both sex, and its identification kit.

Bacterial Isolate		Female		Male		Total	Percent
		Not-diabetic	Diabetic	Not-diabetic	Diabetic		
Gram Negative	*E. coli*	28	12	6	6	52	44.82%
	P. aeruginosa	4	2	0	3	9	7.76%
	K. pneumonia	3	1	3	1	8	6.90%
	E. aerogenes	4	0	0	0	4	3.45%
	E. cloacae	2	0	0	0	2	1.72%
	P. mirabilis	8	2	1	1	12	10.34%
	S. marcescens	0	1	1	0	2	1.72%
	C. freundii	1	0	0	0	1	0.86%
	A. baumannii	0	0	1	0	1	0.86%
Gram Positive	*S. aureus*	4	2	0	0	6	5.17%
	S. pyogenes	1	2	0	0	3	2.59%
	E. faecalis	6	1	0	0	7	6.03%
	S. epidermidis	2	1	0	1	4	3.45%
	B. subtilis	2	0	0	0	2	1.72%
	S. saprophyticus	1	1	1	0	3	2.59%
		56.9%	21.6%	11.2%	10.30%		99.98%

Table 8

Antimicrobial inhibition zone diameter (mm) against isolated bacteria from diabetic and non-diabetic UTI samples

Bacteria Isolate	C_{30}	Na_{30}	Cip_5	Va_{30}	TE_{30}	Ceu_{30}	Cep_{30}	Am_{75}	G_{10}	Amp_2
E. coli	21	22	33	24	9	17	-	22	7	8
P. aeruginosa	-	-	14	9	-	-	-	-	18	7
K. pneumonia	11	7	21	15	8	-	-	24	8	7
E. aerogenes	7	-	25	15	7	-	12	9	17	9
E. cloacae	8	5	10	11	7	-	-	7	7	4
P. mirabilis	8	23	35	32	-	11	-	18	20	9
S. marcescens	7	7	18	12	4	15	4	8	4	7
C. freundii	9	5	15	11	8	4	5	5	5	-
A. baumannii	10	4	10	10	3	5	7	10	4	-
S. aureus	29	19	33	22	10	12	5	18	15	-
S. pyogenes	24	10	29	18	5	8	4	9	12	19
E. faecalis	20	12	27	12	14	-	4	4	9	3
S. epidermidis	30	12	17	22	11	-	-	2	9	7
B. subtilis	24	11	14	11	9	5	4	7	9	10
S. saprophyticus	19	16	19	12	9	-	-	4	12	4

C_{30} : Chloramphenicol 30µg, Na_{30} : Nalidixic 30µg , Cip_5 : Ciprofloxacin 5µg, Va_{30} : Vancomycin 30µg, TE_{30} : Tetracycline 30µg, Ceu_{30} : Cefuroxime 30µg, Cep_{30} : Cephalothin 30µg, Am_{75} : Amoxicillin 75µg, G_{10} : Gentamicin 10µg, Amp_2 : Ampicillin 2µg.

Table 9
Effectiveness percentage of different antibiotics for isolated bacteria from diabetic and non-diabetic UTI samples

Antibiotic	Disc Code	No. of isolated bacteria		Percentage
		Sensitive	Resistance	
Chloramphenicol	C_{30}	89	27	76.72%
Nalidixic	Na_{30}	84	32	72.43%
Ciprofloxacin	Cip_5	97	19	83.62%
Vancomycin	Va_{30}	93	23	80.17%
Tetracycline	TE_{30}	47	69	40.52%
Cefuroxime	Ceu_{30}	34	82	29.31%
Cephalothin	Cep_{30}	31	85	26.72%
Amoxicillin	Am_{75}	61	55	52.59%
Gentamicin	G_{10}	57	59	49.14%
Ampicillin	Amp_2	66	50	56.89%

4.2 Discussion

Bacterial studies of 116 urine samples that introduced in this study indicated that both gram positive and gram negative may cause urinary tract infection with different percentage. There are difference between male and female or diabetic and non-diabetic as in Table 7 .

The samples that have a bacterial count $\geq 10^5$ bacterial cells/ml considered as significant UTI and required treatment with suitable antibiotic according to resistance profile test (Khleifat et al., 2006).

Female patients exhibit a high percentage of UTI-causing bacteria, 56.9% for female non-diabetic and 21.6% for female diabetic as a total of 78.5 % of all patients due to a short urethra and the vagina opening is closed to the anal region (Sibi et al., 2011). While 11.2 % for male non-diabetic and 10.3 % for male diabetic as a total 21.5 % of all patients (Daoud et al., 2011).

In diabetic and non-diabetic samples, *E.coli* have a higher incidence than another UTI-causing pathogenic. *E.coli* as a member of *enterobacteriaceae* with 44.82% give us an evidence that *E.coli* have a fimbriae which facillate them to invade epithelial cells of urinary tract and cause UTI.

As in the most results including our result, *E.coli* is the most prevalent causative bacteria of UTI. This case occur because *E.coli* have different virulence factors (VFs) that facillate them to cause infection. One of these VFs is the presence of adherence organelles fimbriae (Type

1 fimbriae, S fimbriae, P fimbriae and afimbrial adhesion) that increase the chance of adherence of *E.coli* to uroepithelial tissue which is the most important step of UTI (Hoepelman et al., 2003). Another virulence factors are aerobactin, cytotoxic necrotizing factor and hemolysin, that differ in the action which lead to UTI (Daoud et al., 2011).

Proteus mirabilis represented 10.34% of infected sample with UTI with higher percentage in female rather than male. *P. mirabilis* is the second type of bacteria that cause UTI due to expression of four types of fimbriae (as in *E.coli*) that used to adherence step, and the production of hundreds of flagella per cell (peritrichous) that facillate bacterial cells to swim and attacked to the uroepithelial tissue. These flagella-mediated motility is required to ascend the ureters to the kidney and cause UTI (Chen et al., 2012).

Pseudomonas aeruginosa represented a third UTI-causing bacteria with 7.76% in both sex. The resistance of *P. aeruginosa* for antiseptic techniques in the hospitals helps this bacteria to caused UTI-infection. Another reasons that facillate *P. aeruginosa* to cause UTI are presence of capsule that protect them from phagocytosis, and presence of peritrichous flagella that provide motility toward uroepithelial tissue (Mittal et al., 2009).

Klebsiella pneumonia represented 6.90% of infected samples, which is agreement with Daza et al., 2001. This bacteria have the same way for infection as *E. coil* due to the presence of capsule and fimbrae, so it easy to invade and enter urinary tract of both male and female and cause UTI.

Another *Enterobacteriaceae* species exhibit a low percentage of infected sample. *E. aerogenes* (3.45 %), *E. cloacae* (1.72 %), S. *marcescens* (1.72 %), *C. freundii* (0.86 %) and *A. baumannii* (0.86 %). All these bacteria species are pathogenic due to presence of capsule of all except *C. freundii*.

A previous study show-to some extent-the same results as in our study. Khleifat et al., 2006, represent that *E.coli* 53.24% is responsible to the UTI followed by *E. faecalis* and *P. mirabilis* (24.05%, 19.537% respectively). While in our results *E. faecalis* occupied fifth position but in the first line related to gram positive bacteria. however different results have been reported. The similarities and differences in the type and distribution of UTI-causing bacteria may result from different environmental conditions and host factors, and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each community.

In this study, diabetic samples represented 37 samples (31.9%) distributed among 12 male (32.4%) and 25 female (67.6%). All this samples affected with UTI with different bacterial species. These

bacterial species that were isolated from diabetic samples are parts of bacterial species that were isolated from non-diabetic samples.

E.coli represented 50% of diabetic male samples, and 48% of diabetic female samples, which show an equal percent. That is mean that both diabetic male and diabetic female may introduce to the UTI- causing bacteria in the same pattern. *P. aeruginosa* is the second bacterial species that were isolated from diabetic samples which represented 8% of diabetic female and 25% of diabetic male. The high percent of the presence of *P. aeruginosa* in urine sample of diabetic male compared with non-diabetic male (0.0%) due to the immune suppression that happened by opportunistic UTI (Goswami et al., 2001).

E. aerogenes, E. cloacae, C. freundii, A. baumannii and *B. subtilis* are five bacterial species that were not isolated from all diabetic samples in our study. Although there are several studies represented them in both male and female that affected with diabetes mellitus. Because, firstly the number of diabetic samples is low compared with other studies, secondly the percentage of isolation of these species generally low specially gram negative species (*E. aerogenes, E. cloacae, C. freundii and A. baumannii*) (Patterson & Andriole, 1997).

Although The relationship between sugar level and risk of UTI in diabetes is controversial, DM has for a long time been associated with increase in prevalence of bacteria compared with patients without diabetes (Carton et al., 1992). In women case, the prevalence of bacteria is high if the women diabetic, but the diabetic men are more suspected to UTI than diabetic women (Sibi et al., 2011).

According to morphological studies and staining and biochemical test there are no differences in behavior of bacterial species that were isolated from diabetic and non-diabetic samples. As seen in the (Tables 4,5 and 6). So The bacteria causing UTIs in diabetic patients are the same as in UTIs in non-diabetic patients.

At all studies, DM will increased the risk of UTI. This idea related to many biological aspects. The first suggested mechanism in the pathogenesis of the increased prevalence of UTI in diabetic patients is glucosuria that enhanced bacterial growth, by increased the cells number (Hoepelman et al., 2003).

The second mechanism is neutrophils dysfunction. Multi-studies show that polymorphonuclear cells of patients with DM show decrease in number and function (chemotaxis, phagocytosis, killing) of them (Hoepelman et al., 2003). In addition, Local cytokine secretion might be of importance. Cytokines are small proteins, which play an important role in the regulation of host defenses against bacterial infections. Urinary cytokine excretion IL-8 and IL-6 concentrations has been low in diabetic patients than in nondiabetic patients. Lower urinary leukocyte cell count

correlated with lower urinary IL-8 and IL-6 concentrations. This might contribute to the increased incidence of UTIs in this patient group (Bonadio et al., 2004).

The third suggested mechanism for the increased risk of bacteriuria in patients with DM is an increased adherence of bacteria, which can be due to either a decrease anti-adherence activity of the urine, and an enhanced adherence capacity of uroepithelial cells (Goswami et al., 2001). Anti-adherence mechanism done by expression an glycoprotein called Tamm-Horsfall Protein (THP) which produce from kidney and prevent bacterial fimbriae (type 1 and S) from attachment with uroepithelial tissue. This protein level in DM patients was low that enhance adherence of bacteria to uroepithelial tissue and caused UTI (Hoepelman et al., 2003).

All isolated bacterial species , gram negative and gram positive, were treated with various antibiotics in order to selected suitable antibiotic for treating the patients in early stage of UTI. This sensitivity profile checked by disk-diffusion method using different types of antibiotic that belong to different antibiotic families (Khleifat et al., 2006).

Table 8 and 9 show that the sensitivity and resistance level of commonly used antibiotic was differ from one bacterial species to another, and depended on the mechanism of action of these antibiotics.

Gram positive bacteria could be sensitive mainly to Chloramphenicol, which belong to Chloramphenicol family that inhibit the protein synthesis by inhibition of peptidyl transferase enzyme (Stewart & William Costerton, 2001). Ciprofloxacin (fluoroquinolones family) and Vancomycin (glycopeptides antibiotic) were found the most effective antibiotic to all isolated bacterial species in both diabetic and non-diabetic UTI, although both of them were differ in its mechanism, Ciprofloxacin prevent DNA synthesis by inhibition of gyrase and topoisomerase enzymes, while Vancomycin prevents cell wall synthesis by inhibition of *N*-Acetylmuramic acid (NAM) and *N*-Acetylglucosamine (NAG) production (Mansour et al., 2009; Stewart et al., 2001).

Mainly Cefuroxime (cephalosporin family), Cephalothin (cephalosporin family) and Ampicillin (aminopenicillin family) were resistance to the most isolated species, due to presence of β-lactamase enzyme, which attacked with β-lactam ring that found in the structure of these antibiotics. So to use these antibiotics in UTI treatment, should be combined with β-lacamase inhibitor such as clavulonic acid (Takahashi et al., 2004).

Nalidixic acid (fluoroquinolones family), Tetracycline (tetracycline family) and Gentamicin (aminoglycoside family) can't be used for all isolated species because these antibiotic not sensitive from all isolated

species. *K. pneumonia* is sensitive to Amoxicillin that belong to β-lactam antibiotic family, that is mean that β-lactamase in this case not effective and Amoxicillin stay active (Gul et al., 2004). Augmentin is a combination between Amoxicillin and clavulonic acid that used widely range to treat UTI in both diabetic and non-diabetic , the combination between them to prevent interaction between β-lactamase enzyme and β-lactam (Stewart et al., 2001).

The present study shows that diabetic and non-diabetic UTI pathogens decrease susceptibility to the most types of antibiotics, so it is very necessary to develop new antimicrobial and therapeutic agents that have high effectiveness with no side effect, easy availability and also less expensive.

4.3 Conclusions

As conclusion, *Escherichia coli* is the most common bacterial species that cause urinary tract infection in both diabetic and non-diabetic patients. Generally, gram negative bacteria (specially that belong to *enterobacteriaceae* family) is the most common UTI-causing bacteria than gram positive bacteria .

There are no significant differences between the same bacterial species whether isolated from diabetic urine sample or from non-diabetic urine sample. But the counting bacterial cells in the diabetic sample is more than that from non-diabetic sample.

UTI patients can be treated with different types of antibiotic as Ciprofloxacin regardless of male or female, single or married, diabetic or not. But should be taken in our mind if the patients are pregnant or not (if female) and child or adult or aged.

4.4 Recommendations

There are many questions remain un-answered, is there any genetically variants of the same bacterial species that facilitate and promote the highly growth in the diabetic urine sample compared with non-diabetic urine sample?. And is there any growth determents of the bacterial cells in diabetic urine sample?

So we recommended for further genetically studies to detect the reasons that facillate growth of specific types of bacteria with highly yield and inhibit another.

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